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Enhancement Antifungal Activity And Mechanical Properties Of Pickle Leather Composited With Silver Nanoparticle

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Abstract

The red betel leaves has tannin active compound. Biosynthesis of silver nanoparticles was utilized tannin compound that could change metal ion to atom. Modification of pickle goat leather was modificated by using silver nanoparticle and methyltrimethoxysilane (MTMS). The objective of this study was to determine the characteristic of goat pickle leather include antifungal activity and mechanical properties. The test for antifungal activity against Candida albicans ANCC 0048 was conducted by diffusion method. For the statistical testing, samples were analysis by using the ANOVA and LSD test. For the mechanical properties test, the samples were measured by a tensile strength tester. The result of antifungal activity showed that there was a significant difference between the interaction of time incubation and sample variation. Leather after composited with nanoparticle showed the highest antifungal activity against Candida albicans ANCC 0048 and the biggest in toughness. The result of mechanical properties showed that Leather - NAg was hard and tough with tensile strength of 9.32 MPa and elongation at break of 33.47%. However, leather after composited with MTMS and silver nanoparticle has the lowest antifungal activity but the highest modulus Young

Keywords: *Antifungal activity, mechanical properties, pickled goat leather, and silver nanoparticle*

1 Introduction:

The textile industry in Indonesia experienced a significant increase in the first quarter of 2019 as much as 18.98% compared to 2018 which only reached 7.46% [1]. The most widely used textile raw material is derived from the livestock skin because it is easily obtained, flexible, and durable. Goat leather is widely used in the industrial sector compared to sheepskin and cowhide because it has the characteristics of shiny leather and the processing is relatively simple [2].

Raw goat leather is easily damaged or deformed, so the pre-tanning stage is needed to be done to preserve the skin by the pickling process. The principle of the pickling process is to change the skin under acidic conditions around pH 2.5-3 with a liquid consisting of salt (NaCl), acid, and water. The acids used include H_2SO_4, HCl, and HCOOH [3]. The pickle skin produced is still in the form of raw material, it needs to be modified with silver nanoparticle and the addition of *methyl trimethoxy silane* (MTMS) compounds to control the growth of microorganisms that can accelerate the damage and reduce mechanical strength in the skin [4].

Nanoparticles are materials in the size of 1-100 nm which have unique properties related to the shape, size and distribution of particles in the determination of optical, catalyst, biomedical, and antimicrobial properties [5]. There are various types of nanomaterials such as copper, zinc, titanium, magnesium, gold, alginate, and silver [6]. Based on a study by Chamakura *et al*. [7] among these metals, silver is known to have compatible properties and the best mechanism of action as an antimicrobial because there are no microbes that are resistant to silver. Synthesis of silver nanoparticles can be carried out in two methods, there are top-down and bottom-up methods. Chemical reduction methods are often used because the process is fast and easy but it is feared that it can have a negative impact on the environment. These problems can be minimized by applying the principle of biosynthesis to the chemical reduction method by using plant extracts [8, 9], such as red betel leaf (*Piper croatum*) as a reducing agent.

Plant extracts which contained in red betel leaves are phenolic compounds in the form of tannins that have biological ability to precipitate proteins on microbes and have a strong tendency to reduce charged metal ions (Ag^{+}) to nanoparticles (Ag^{0}) [10]. Silver nanoparticles can inhibit the growth of eukaryotic microorganisms that are pathogenic, one of which is a fungus *Candida albicans* [11]. This fungus is often called "yeast-like fungi" usually found in the human body in the skin, mucous membranes, and genitalia in women which can cause superficial infections of the skin [12].

Textile products that are being developed in addition to antimicrobials are self-cleaning textiles that have hydrophobic properties that cause dirt to be easily released when doused with water. Hydrophobic properties can be obtained by modifying the surface of the leather by coating one of the silane compounds, one of them is the MTMS compound. MTMS compounds have a compound formula of $CH_3Si(OCH_3)_3$. The existence of covalently bound methyl substituents can reduce surface energy making the surface is hydrophobic [13]. This study aims to determine the characteristics of modified pickled goat leather by using silver nanoparticles and MTMS compound including antifungal activity against *Candida albicans* and the mechanical properties of the leather.

2 Experimental

2.1. Materials

The pickled goat leather was purchased from the leather industry in Yogyakarta, Indonesia. Salt of silver nitrate, salt of natrium chloride, starch powder, ethanol, and methyl trimethoxy silane were purchased as commercial products. Potato Dextrose Agar (PDA) and Potato Dextrose Broth (PDB) were purchased from Oxoid. *C. Albicans* ANCC 0048 was obtained from the collection in Faculty of Mathematics and Natural Sciences, Yogyakarta State University, Indonesia.

2.2. Methods

The study was conducted in consecutive stages as follows: extraction of *piper betle* leaf, preparation of silver nanoparticle, deposition of silver nanoparticles on pickled goat leather, characterization included testing of antifungal activity and mechanical properties.

2.2.1. Preparing of Solution: The $AgNO_3$ solution is prepared by dissolve of 0.17 grams of $AgNO_3$ powder in a 1000 mL of distilled water. The 0.05% of starch was prepared by dissolving 0.50 grams of starch powder in 1000 mL of distilled water and homogenized. As much as 3% of MTMS solution was prepared by dissolving of 30 mL of MTMS in a 1000 mL ethanol of 96% and homogenized.

2.2.2. Red Betel Leaf Extraction: Red betel leaves are washed and then are dried. After that, as much as 25 grams of the leaves are weighed and then soaked it in 250 mL of distilled water and then heated for 10

minutes at a temperature of 80° C. The cooled mixture is then filtered by using Whatman No.42 filter paper to obtain a red betel leaf extract.

2.2.3. Biosynthesis of Silver Nanoparticles: A total of 40 mL of red betel leaf extract was soaked into a 500 mL beaker glass and 260 mL of silver nitrate solution was added. The mixture is allowed to stand for 2 hours at room temperature. Subsequently, 100 mL of starch solution was added to the mixture and homogenized using a shaker for 2 hours. The mixture formed is allowed to stand for 72 hours to form colloidal silver nanoparticles.

2.2.4. Deposit of Silver Nanoparticles on Pickled Goat Leather: Pickled goat leather is cut to size 20 cm x 20 cm and then cleaned using distilled water and dried at room temperature. Pre-prepared pickled goat leather is then soaked in distilled water for 24 hours to obtain goat leather as a control (C0). The deposit of silver nanoparticles was carried out by soaking the leather of the goat in an Erlenmeyer containing 100 mL of colloidal silver nanoparticles and shaker for 24 hours at a speed of 155 rpm. The leather is dried for 24 hours to obtain a sample (Leather - NAg).

2.2.5. Modification of Pickled Goat Leather with MTMS: Goat leather is immersed in 3% of MTMS solution and shaked for 30 minutes to produce a leather sample (Leather - MTMS). Another variation is modifying goat leather by adding silver nanoparticles and MTMS 3%. The leather is soaked in a colloidal silver and putting it in a shaker for 3 hours then added 3% of MTMS then shake again simultaneously for 24 hours. The resulting leather is sample leather (Leather - NAg - MTMS). The same stage is performed on the leather with the addition of 3% of MTMS followed by the addition of silver nanoparticles then it will be obtained leather sample (Leather - MTMS - NAg).

2.2.6. Antifungal Activity Test: Antifungal activity test was carried out by the diffusion method against the *C. Albicans* ANCC 0048. The fungus was cultured on the Potato Dextrose Broth medium for 24 hours. Fungus suspension was inoculated on PDA media in ½ part of the petridish by the spread plate method. Variations in the sample of goat leather (Leather (C0), Leather - NAg, Leather - MTMS, Leather - NAg - MTMS, Leather - MTMS - NAg) are cut using a paper hole puncher and then sterilized for ±5 minutes in ethanol of 70%. Samples were placed on a fungus culture and then incubated at 37°C. Inhibition zone diameters are measured for every 24 hours for 96 hours.

2.2.7. Mechanical Test: Samples of pickled goat leather are cut like a dumbbell specimen with SNI 06-1795-1990. The machine used for tensile strength testing is the Jester Tensile Strength Meter with Lod cell 2000 kgf and Gauge length 50 mm. Mechanical properties testing was carried out according to SNI 06-1795-1990 standards. The test is carried out by pulling the specimen to the breaking point [14]. The tensile strength test will produce a relationship curve between the magnitude of the force with the displacement expressed in the Stress-Strain Curve.

2.2.8. Data Analysis: Characteristics of modified pickled goat leather include antifungal activity and mechanical properties were analyzed using IBM SPSS Statistics 25. The Analysis of Variance (ANOVA) test is used to study the significant different among characteristic of all leather sample and the Least Significant Different (LSD) test is used to study the significant different between two leather sample. The results of mechanical properties test are then made into Stress-Strain Curve by plotting tensile strength against elongation (%) to determine the toughness and ability of leather in withstand the load of material.

3 Results and Discussion

3.1. Biosynthesis of Silver Nanoparticles

Silver nanoparticles were synthesized by the principle of biosynthesis using red betel leaf extract as a bioreductor. The biosynthesis process is carried out by mixing 0.001 mol/L $AgNO_3$ solution, red betel leaf extract, and 0.05% of starch solution. The volume ratio of $AgNO_3$: leaf extract : starch was 6.5 : 1 : 2.5. Colloidal silver nanoparticles has showed a color change from brownish red to blackish brown on 3 days (**Figure 1**). The Particle Size Analyzer (PSA) test result showed the size distribution of silver nanoparticles between 52.9 nm - 223.0 nm with an average particle size of 95.1 nm. Thus silver nanoparticles were successfully synthesized from silver nitrate solution using red betel leaf extract as a bioreducing agent.

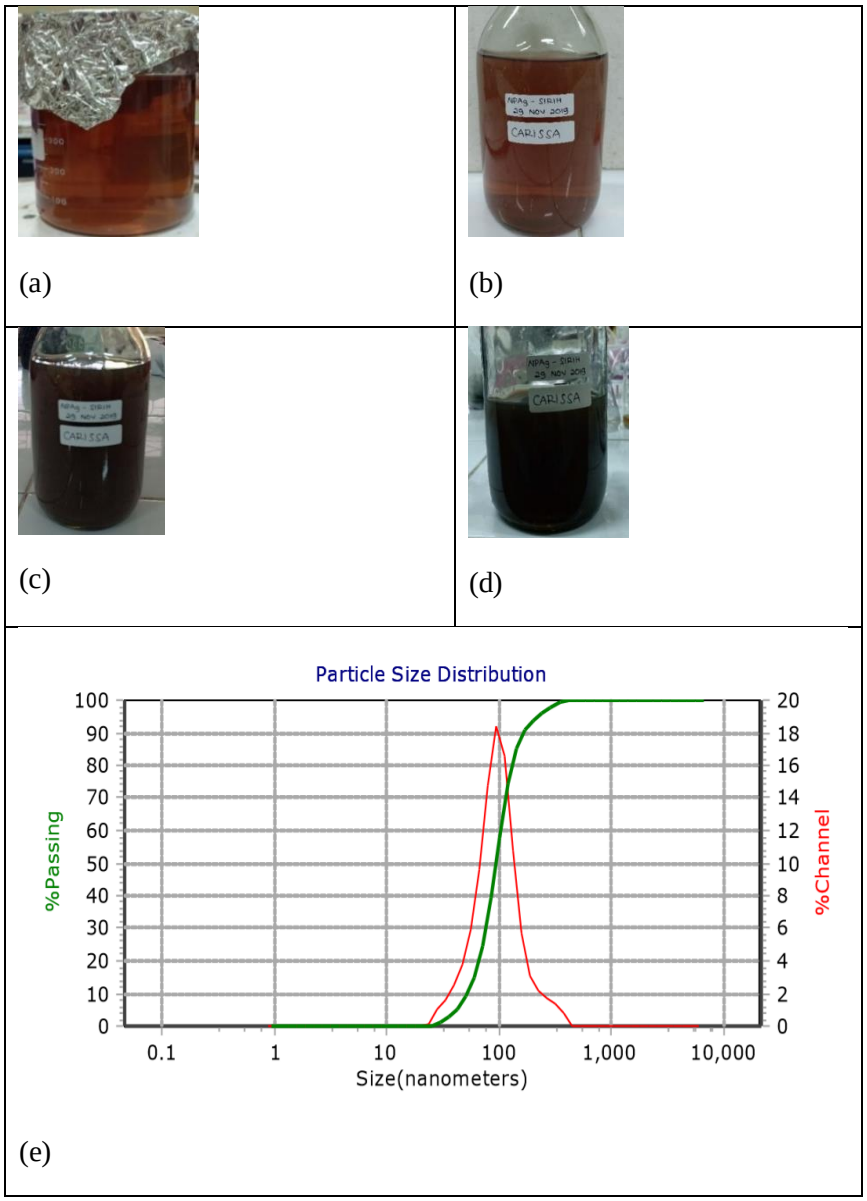
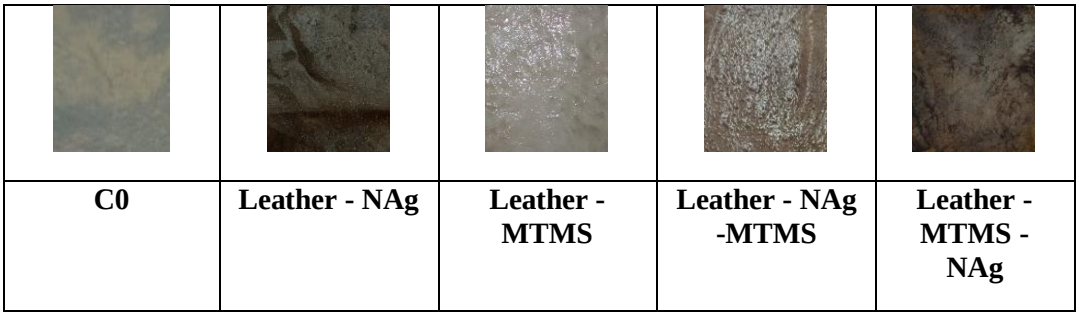


Figure 1. Colloidal Silver Nanoparticles (a) 1 Hours, (b) 2 Hours, (c) 24 Hours, (d) 72 Hours, (e) Particle Size Distribution

The color change indicates the process of reducing Ag^+ to Ag° [15]. The reaction is slow and is an equilibrium reaction. The formation reaction silver nanoparticle shown as $4\ Ag^+(aq) + 2\ H_2O(l) \leftrightarrow 4Ag°\ (s) + O_2(g) + 4H^+(aq)$.

3.2. Modification of Pickled Goat Leather

Modified pickled goat leather shows physical differences in color differences and stiffness of leathers (**Figure 2**). The color change indicates that silver nanoparticles and silane compounds have been successfully deposited into collagen fibers to modify the surface of the leather. Collagen fibers in leather are composed of amino acids that have amine ($-NH_2$) and carboxyl (-COOH) functional groups [16]. Silver atom (Ag) can interact with electrons of C=O and NH groups so that the surface of the leather is coated with silver nanoparticles [17].



C0	Leather - NAg	Leather - MTMS	Leather - NAg -MTMS	Leather - MTMS - NAg

Figure 2. Images of Pickled Goat Leather Before and After Modification

The coating of the silver nanoparticle and MTMS compound showed difference in color and rigidity compared with pickled leather before modification (C0). Leather after modification with adding MTMS had more rigid than leather before modification. There may be caused a strong hydrogen bond between the -NHCOO functional group in leather and the Si-OH in MTMS which affected the fiber stabilization as in **Figure 3**.

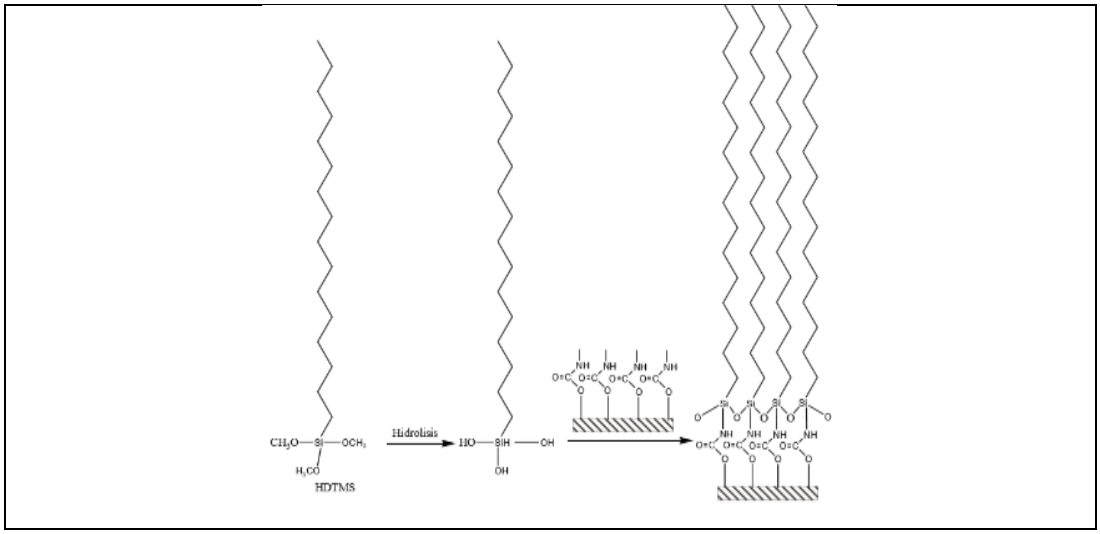


Figure 3. The Mechanisms of Hydrophobicity on Spandex by Silane Compounds [18]

3.3. Antifungal Activity of Leather

The results of the average measurement of the inhibition zone diameter of the *Candida albicans* ANCC 0048 can be seen in **Table 1**.

Table 1. The Inhibition Zones of Leathers against Candida albicans ANCC 0048

Sample of Leather	Inhibition Zone Diameter (mm)			
	24 hours	48 Hours	72 hours	96 hours
Leather	0.29	0.21	0.24	0.21
Leather – NAg	0.48	0.42	0.38	0.35
Leather – MTMS	0.08	0.10	0.14	0.00
Leather - NAg – MTMS	0.30	0.25	0.30	0.19
Leather - MTMS – NAg	0.08	0.09	0.10	0.00

Based on **Table 1**, Leather - NAg shows the highest antifungal activity. The adding of MTMS can decrease antifungal activity of leathers. The adding of combination of MTMS and silver nanoparticle simultaneously can decrease antifungal activity of leathers also. Thus, only the adding of silver nanoparticle can increase ability of leather in inhibiting the growtth of fungi. This relationship between the diameter of the inhibition zone (mm) and the incubation time (hours) could be seen in **Figure 4**.

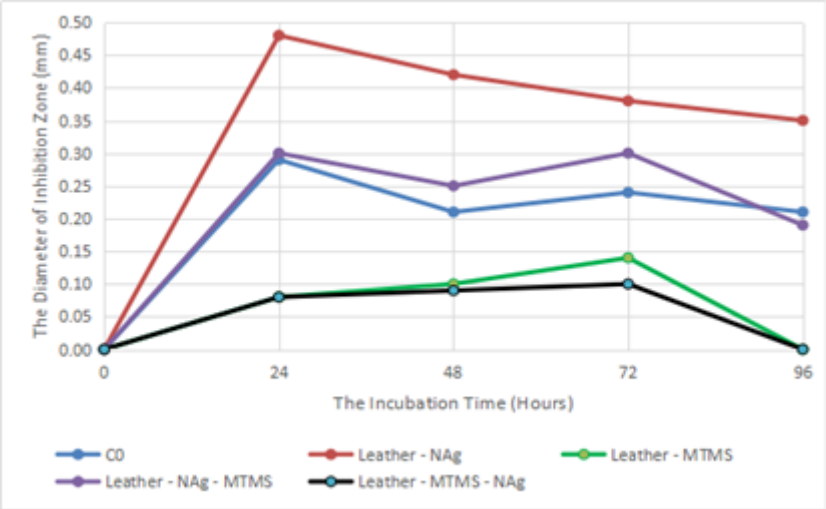


Figure 4. The Inhibition Zone of Leather against Candida albicans ANCC 0048

Microbes have 4 growth phases, included the lag phase, the log phase, the stationary phase, and the death phase. The lag phase is the phase of adjustment between microbes and their environment with no increase in the number of cells. This phase can take place quickly depending on the nutrition of the media and the type of microbes. The exponential phase is characterized by rapidly increasing growth. Based on **Figure 4,** it is known that the maximum inhibition of leather sample in repressing growth of ***Candida albicans* ANCC 0048** occurs at 24 hours. The addition of silver nanoparticles can increase antifungal activity of leather. The Leather - NAg and Leather - NAg - MTMS samples have the bigger inhibition activity in inhibiting the growth of the *Candida albicans* ANCC 0048 than the leather without modification (C0). Silver nanoparticles can interact with -SH groups contained in cell membranes and also with DNA that caused DNA structure to be disrupted so the microbes cannot replicate itself and eventually microbial cells died [19].

All leather samples showed inhibition activity, however Leather - NAg samples had the highest ability to inhibit ***Candida albicans*** growth at each incubation time. However, modification leather with adding MTMS can decrease antifungal activity of leather. Interaction between funtional groups of MTMS and functional groups of leather is very strong and MTMS can coat surface of leather significantly, this can

cause barrier for interacting leather with nanoparticle of silver, as a result leather after modification with MTMS has decreased in antifungal activity. The addition of silane compounds in Leather - MTMS also gave the ability to inhibit the growth of fungi, but the inhibitory zones that are formed are relatively small. Silane compounds can inhibit the growth of microbes due to properties similar to detergents that are hydrophilic and hydrophobic [20].

The Leather - MTMS - NAg samples have the lowest an antifungal activity. This is due to the silver nanoparticles were deposited after the leather was coated with MTMS compounds so the silver nanoparticles could not completely coat and interact with the leather surface.

3.4. Statistical Test of Antifungal Activity

The diameter of inhibition zone is analyzed by statistical tests using Analysis of Variance and Least Significant Different. The results of ANOVA test are shown in **Table 2**. The ANOVA test is used to determine whether there is an effect of incubation time, leather sample variation, and interaction between an incubation time and leather sample variation on antifungal activity. The LSD test is used to determine the significance difference in antifungal activity between two leather samples.

Table 2. The Results of ANOVA Test : The Effect of Incubation Time and The Type of Leather Sample toward Antifungal Activity of Leather

Source of Data	**Sum**	**Df**	**Mean Square**	**F**	**Sig.**
Incubation Time	0.084	3	0.028	21.372	0.000
Leather Sample	1.479	9	0.166	126.813	0.000
Incubation Time*Leather Sample	0.176	27	0.007	4.957	0.000

The result of ANOVA test in **Table 2** shows the significant value of 0.000 (sig $\leq$ 0.05) each for the effect of incubation time, the effect of leather sample variation, and the interaction between an incubation time and sample variation against antifungal activity of leather against *Candida albicans*. The result of sig. $\leq$ 0.05 can be interpreted that incubation time, leather sample variation, and interaction between incubation time and sample variation give a significantly different effect on antifungal activity of leather.

The result of LSD test to study the differences in activity between two sample against *C. Albicans* ANCC 0048 is shown in **Table 3**. Between leather without modification and the leather - NAg, leather without modification and the leather - MTMS, leather without modification and the leather - NAg - MTMS, and also between leather without modification and the leather - MTMS - NAg show significant differences against growth the *C. Albicans* ANCC 0048.

Table 3. Interpretation of LSD results to C. Albicans ANCC 0048

The Leather Sample	Conclusion
Leather with Leather – NAg	Significant
Leather with Leather - MTMS	Significant
Leather with Leather - NAg - MTMS	Significant
Leather with Leather - MTMS - NAg	Significant

Based on **Table 3**, it can be concluded that all the leather after modification showed the ability to inhibit the growth of *Candida albicans* significantly different compared to the leather without modification. This reinforces the ANOVA test results that among all samples showed different antifungal activities. Likewise, when we compare the two samples, they show different antifungal activities too.

3.5. Mechanical Properties of Leather

The effect of modification on the leather by adding silver nanoparticle and MTMS toward the mechanical properties of goat leather can be known by determining the tensile strength, elongation at break, and modulus Young. Tensile strength is expressed in units of kgf/cm^2 and then converted to SI units of MPa (1 kgf/cm^2 = 0.098 MPa). The results of testing the mechanical properties of the pickled goat leather are shown in **Table 4**. The mechanical properties of pickled goat leather without modification and with the modification shows different physical properties based on the toughness (ductility) of a material, the order of toughness of variation of the goat leather samples are Leather - NAg> Leather - MTMS> Leather> Leather – MTMS - NAg> Leather – NAg - MTMS.

Table 4. The Mechanical Properties of Pickled Goat Leather

Sample	Elongation (%)	Tensile strength (MPa)	Modulus Young (MPa)
Leather	36,34	3,46	9,52
Leather - NAg	33,47	9,32	27,83
Leather - MTMS	52,30	3,43	6,57
Leather - NAg - MTMS	22,59	0,35	1,57
Leather - MTMS - NAg	2,84	1,88	66,12

Based on the measurement results in **Table 4**, it can be described the Stress-Strain curve, which is the relationship between tensile strength (MPa) and elongation (%) of sample variations as shown in **Figure 5**. Stress is defined as force per unit area (σ) while a strain is defined as the percent of length changes (% ε).

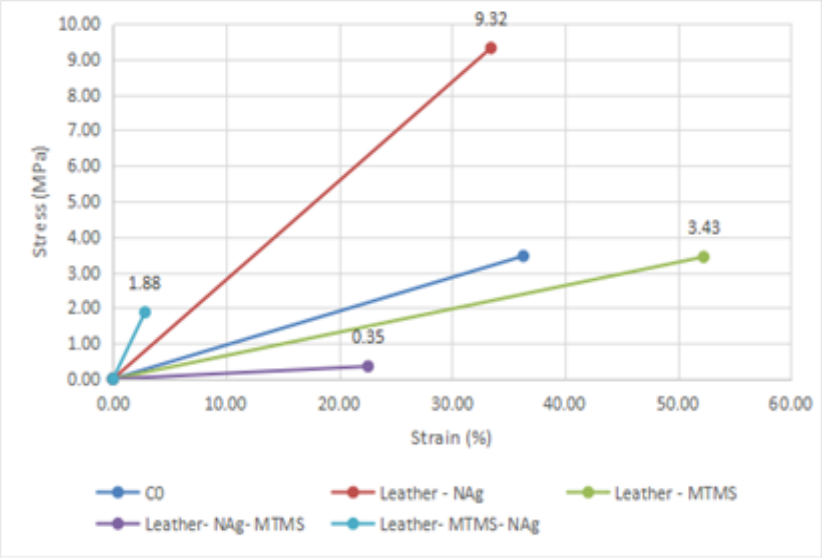


Figure 5. The Stress-Strain Curves of Pickle Leather Material

A polymer has different physical properties, there are brittle, ductile, and elastomer (highly elastic) materials [20]. The test results show that the leather sample (Leather - NAg) is hard and strong material. The leather – NAg is strong in holding the load (hard) and resilient (tough) shown by the area under the largest curve.

The leather – MTMS has elastic properties due to stretching in very long plastic areas. The value of length changes is used to determine the toughness of the material. Material with a value of small or near zero elongation (%) is brittle [21]. The leather – MTMS - NAg sample is rigid and easily broken (brittle) because it only has a slight stretching in the plastic area, resulting in a very small elongation value (%). The sample treatment with the addition of silver nanoparticles followed by the addition of silane compound (Leather – NAg - MTMS) has soft and weak properties shown in **Figure 5** because it has a very small area of the under curve.

4 Conclusion

The silver nanoparticle has been synthesized successfully by using the extract of red betel leaf and starch as stabilizer. The average particle size of nanoparticles was 95.1 nm. The samples of pickled goat leather showed significant differences in inhibiting the growth of the *Candida albicans* ANCC 0048. The leather after impregnation with adding nanoparticle has the highest antifungal activity and the best toughness. The order of toughness of the pickled goat leather samples was Leather - NAg> Leather - MTMS> Leather> Leather – MTMS - NAg> Leather – NAg - MTMS. However, the leather with adding MTMS has the highest in elongation at break. Leather - MTMS - NAg has the highest modulus Young but the lowest antifungal activity against *Candida albicans* ANCC 0048. The leather - NAg was strong and resilient with tensile strength values of 9.32 MPa and elongation of 33.47%.

Acknowledgments

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